Essential Oils and Their Constituents XXVIII. Examination of Oil of Cardamom by Gas Chromatography

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Essential oil of *Elettaria cardamomum,* Maton var. *minuscula,* Burkhill was investigated by gas chromatographic analysis of fractions obtained by distillation under reduced pressure and column chromatography. The presence of limonene, sabinene, cineole, α-terpineol, terpinyl acetate, and borneol, reported by earlier investigators, was confirmed. In addition, the oil was found to contain the following previously unreported constituents: α-pinene, myrcene- *p*-cymene, methyl heptenone, linalool, linalyl acetate, β-terpineol, geraniol, nerol, neryl acetate, nerolidol, and heptacosane. Two ketones tentatively characterized as 2-undecanone and 2-tridecanone also were detected in trace amounts.

OIL OF CARDAMOM derived from *Elettaria cardamomum,* Maton var. *minuscula,* Burkhill (fam. *Zingiberaceae*) has been the subject of several studies which established the presence of the following constituents: α-terpineol, α-terpinyl acetate, cineole, limonene, sabinene, borneol, and terpin hydrate (1–7). The oil is used widely by the pharmaceutical industry, and standards have been issued for a number of medicinal preparations (8–13). The present investigation was undertaken to examine the composition of the essential oil in greater detail and assemble experimental data which should prove of value for the recognition of genuine products.

EXPERIMENTAL

Analytical Sample.—The essential oil was made available by S. H. Kelkar and Co., Bombay, India. It was prepared by steam distillation of seeds grown in the State of Mysore. Product yield was 4.3%. The oil possessed the following physicochemical constants: d_{20}^{20} 0.9334; n_D^{20} 1.459; α_D + 21.2°; acid value, 3.1; saponification value, 91.6; saponification value after acetylation, 120.7; carbonyls as $C_{10}H_{16}O$ (by oximation), 2.8%.

Rectification of Analytical Sample.—The essential oil (660 Gm.) was subjected to fractional distillation employing a column (2 ft. long) packed with single turn glass helices (4 mm. diam.). A reflux-take off of 10:1 was maintained throughout the distillation. Six cuts were made, as shown in Table I.

Chromatography of Fraction 5.—An aliquot (10 Gm.) was chromatographed using 200 Gm. of Brockmann grade I alumina and eluted successively with hexane, benzene, and ether. (See Table II.)

Gas Chromatography of Essential Oil and Isolated Fractions.—Apparatus and general procedures have been described (14). Reoplex 400 (20%) deposited on acid washed Chromosorb W (60–80 mesh) was used as column packing. Fraction 1 (monoterpenes) was analyzed at 110°. All other fractions were examined at 170°.

Identification of Constituents.—Constituents were identified by the serial dilution technique and by comparison of the infrared spectra of isolates with those of authenticated reference standards or published data. For spectroscopic examination, effluent peaks were collected in carbon tetrachloride and purified by rechromatography under optimal conditions of temperature and carrier gas flow rate. Percentages of constituents were calculated by the method of Bartlet, Smith, and Levi (15, 16).

Examination of Residue.—When diluted with ethanol and cooled to 5°, the semisolid residue deposited a white waxy substance which was purified by chromatography using grade I alumina (eluant: *n*-hexane) and repeated crystallization from ethanol, melting point of product 61.5°.

RESULTS AND DISCUSSION

The gas chromatogram of the essential oil is reproduced in Fig. 1A. In addition to the major constituents identified, a number of minor and trace components were characterized, including terpene esters, alcohols, and ketones.

α-Pinene and Sabinene.—The presence of these terpenes was detected in fractions 1 and 2; the former was richer in α-pinene, the latter in sabinene.

Myrcene and Methyl Heptenone.—These constituents were identified in fraction 1 only.

1:8-Cineole and Limonene.—The presence of these constituents was detected in fractions 1, 2, and 3; fraction 2 was richest in both compounds.

p-Cymene.—Small quantities of this terpene were detected in fractions 2 and 3.

Linalool and Linalyl Acetate.—Both of these compounds were present in fractions 3 and 4.

Borneol and β-Terpineol.—Trace quantities of these alcohols were detected in fraction 4.

α-Terpinyl Acetate.—α-Terpinyl acetate formed the major portion of subfractions 5-A and 5-B, and also was present in fractions 3 and 4.

Neryl Acetate.—Small quantities of this ester were found to be present in fractions 5-B and 5-C.

α-Terpineol, Geraniol, Nerol, and Nerolidol.—These alcohols were identified in subfraction 5-C. Traces of α-terpineol were present in fraction 4.

Heptacosane.—Infrared analysis of the white waxy substance, isolated from the distillation residue (Table I), showed it to be a saturated hydrocarbon. On the basis of its melting point (61.5°), the hydrocarbon was identified as heptacosane.

Unidentified Compounds.—Two of the unidentified compounds present in fraction 5-B were found to be ketones. Only trace amounts, 0.1 and 0.2%, respectively, were isolated. Their infrared spectra resembled closely those of 2-undecanone and 2-tridecanone, respectively.

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TABLE I.—FRACTIONAL DISTILLATION OF OIL OF CARDAMOM

Fraction No.	Wt., Gm.	Pressure, mm.	Temp., °C.	n_D^{20}	α_D	%, w/w
1	31.7	90	70–80	1.444	+4.3°	4.8
2	291.9	90	80–105	1.460	+35.0°	44.2
3	61.1	55	95–115	1.466	+17.8°	9.3
4	63.6	12	104–106	1.466	+45.0°	9.6
5	162.2	12	106–130	1.466	+53.0°	24.6
Residue	28.4	...	...	...	...	4.3
					Recovery	96.8

TABLE II.—COLUMN CHROMATOGRAPHY OF FRACTION 5

Fraction No.	Eluant	Vol., ml.	%, w/w
5-A	Hexane	250	73.6
5-B	Benzene	250	5.4
5-C	Ether	1000	19.1

The composition of the oil, deduced by correlation of distillation and chromatographic data, is shown in Table III. Terpin hydrate, found to be present in the oil according to one of the earliest reports, was not detected in any fraction of the oil. It probably had originated from α-terpineol, as suggested by Guenther (7). Terpinene, 1-terpinene-4-ol, and its esters, formate and acetate, known to be present in

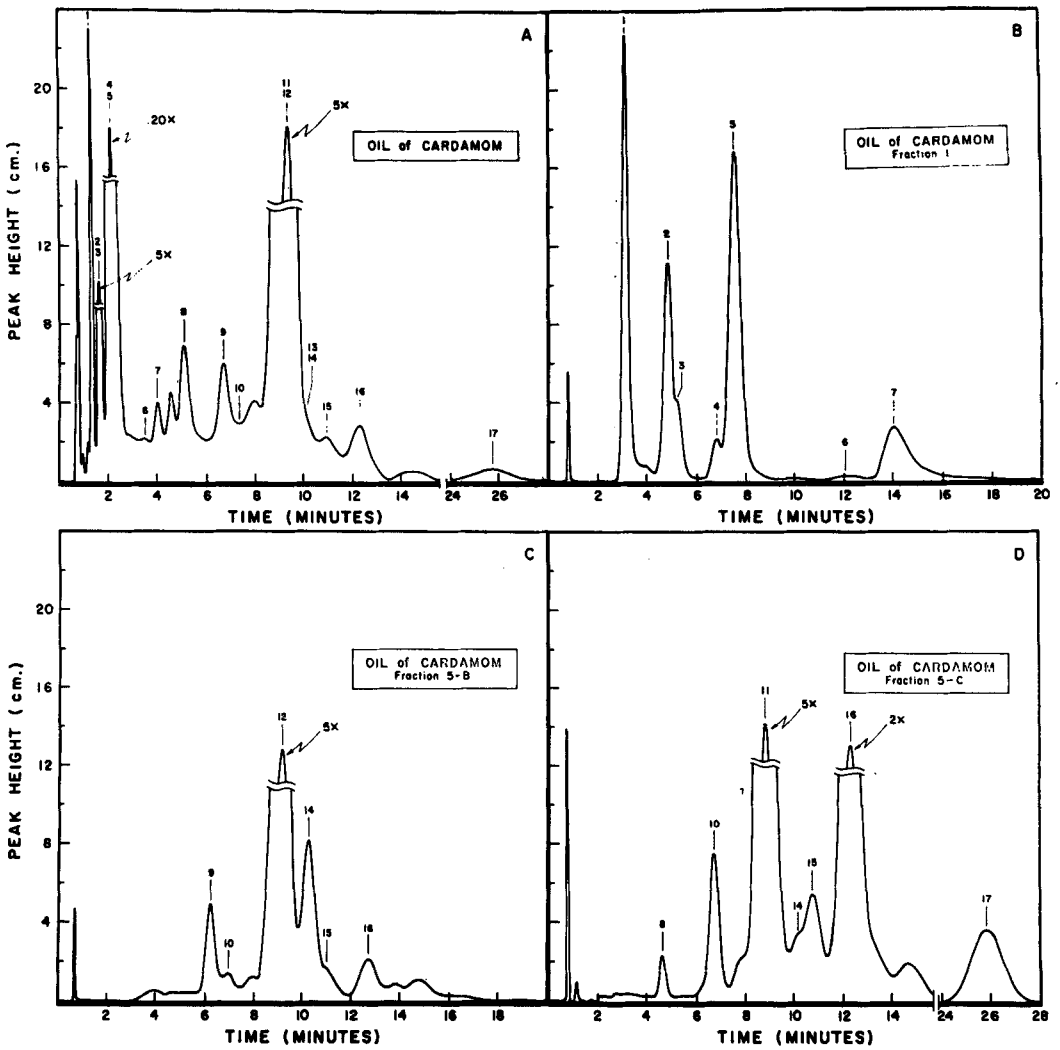

Fig. 1.—Gas chromatographic examination of oil of cardamom and its fractions. Column: Reoplex 400 on Chromosorb W (20%); temperature: A, C, and D: 170°C.; B, 110°C. Helium: 75 ml./min. Sample size: A, 8 μl.; B, 1.5 μl.; C, 5 μl.; D, 3 μl.

TABLE III.—COMPOSITION OF OIL OF CARDAMOM

Peak No.	Constituent	Retention Time[a] (a)	(b)	%, w/w
1	α-Pinene	0.44	0.25	1.9
2	Sabinene	0.71	0.32	4.5
3	Myrcene	0.77	0.32	0.2
4	Limonene	1.00	0.41	14.3
5	Cineole	1.11	0.41	30.7
6	*p*-Cymene	...	0.68	1.9
7	Methyl heptenone	2.05	0.78	0.8
8	Linalool	...	1.00	0.9
9	Linalyl acetate	...	1.12	1.2
10	β-Terpineol	...	1.45	0.8
11	α-Terpineol	...	1.84	3.7
12	α-Terpinyl acetate	...	1.84	28.1
13	Borneol	...	1.95	0.1
14	Neryl acetate	...	1.95	0.3
15	Geraniol	...	2.38	0.7
16	Nerol	...	2.42	1.4
17	Nerolidol	...	5.04	0.3
18	Heptacosane	...	...	0.5
	Unidentified compds.	...	...	7.7

[a] Experimental conditions: (a) temperature, 110°C.; reference standard, limonene; (b) temperature, 170°C.; reference standard, linalool.

oil derived from the Ceylonese cardamom, *E. cardamomum* var. *β-major* Thwaites (17) also were absent in the oil investigated. Therefore, gas chromatographic examination can be applied conveniently to distinguish between the oil of cardamom derived from var. *minuscula* Burkhill and that derived from var. *β-major* Thwaites.

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Potentiation of the Antimicrobial Activity of Bithionol

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Lauricdiethanolamide potentiates the antimicrobial activity of bithionol. This is probably due to the increased solubility of the bithionol in the cellular fluids and to the decreased surface tension which permits a more rapid diffusion of the phenolic compound through the cell wall. This compound also is antagonistic to the phenylmercuric compounds. The antimicrobial activity of bithionol is potentiated by the presence of phenylmercuric ion. This potentiation does not change when the ratio of phenylmercuric ion is changed over a range of 0.1–0.5 parts to 1 part bithionol. Solutions of these compounds were found to be stable when stored at room temperature for 1 year.

THE EMERGENCE of antibiotic resistant microorganisms has brought about a renewed interest in some of the older antimicrobial agents (1–3). The phenolic compounds, the quaternary ammonium compounds and the mercurials have all been reevaluated by different groups in recent years.

The two compounds most worked with are bithionol [2,2′-thiobis(4,6-dichlorophenoxide)] which was first reported by Muth (4) in 1933 and hexachlorophene [2-2′-methylenebis(3,4,6-trichlorophenol)]. These compounds are bacteriostatic agents which are commonly employed in 2% concentrations in soaps and topical solutions (6). These agents, when used over a long period of time, gradually impart a residual activity to the treated area; however, since they are only bacteriostatic in action, they leave much to be desired in the search for a good anti-infective.

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The phenylmercuric compounds are bactericidal in nature, but they do not have any residual activity. Barker (5) reported that some cationic and some anionic agents such as surfactants tend to inactivate these compounds, and thus they cannot be combined with soaps or detergents which would also serve as good mechanical cleansers.

The quaternary ammonium compounds, such as benzalkonium chloride and cetylpyridinium chloride, are inactivated by anionic agents, by serum, and by some of the fats on the skin. These compounds are also incompatible with the phenolics and the phenylmercuric compounds.

This work was undertaken to find, if possible, a nonionic agent which would be compatible with bithionol and the phenylmercuric compounds and also to seek a combination of antimicrobial agents that would give a rapid killing effect and also have a residual activity.